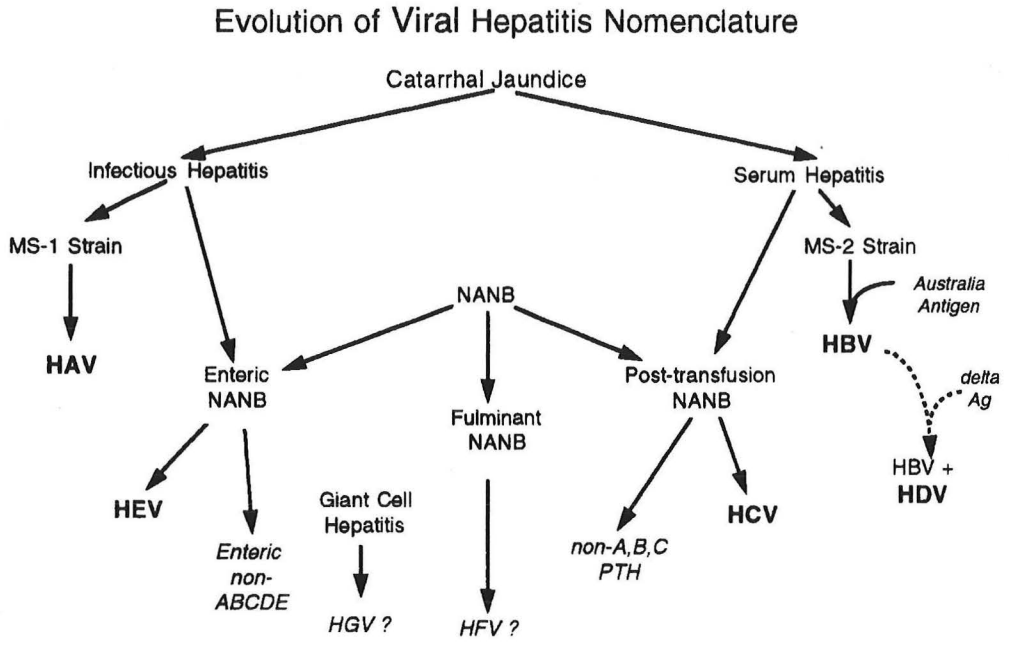

Seronegative (non-ABCDE) Hepatitis

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History:

Outbreaks of jaundice were described as early as the 5th century B.C. and the writings of Hippocrates include the term "epidemic jaundice" (1-3). Epidemics of "campaign jaundice" have been reported to afflict armies from the Middle Ages up to and through the 20th century. As early as the 1850's, epidemics of hepatitis were associated with vaccines containing materials of human origin (4). However, a general appreciation of distinct forms of hepatitis did not occur until the World War II era when epidemiologic studies first suggested the existence of both a fecal-oral and parenteral routes of transmission for what previously had been termed "catarrhal" or "common infective" jaundice (2,3). From the 1940's through the mid-1970's, acute viral hepatitis was believed to result from infections by two and only two distinct viruses. Infectious hepatitis was believed to be a manifestation of an enteric "hepatitis A" infection which was designated as the MS-1 strain and was initially clearly distinguished from a second MS-2 strain of serum or "hepatitis B" by the studies of Saul Krugman at the Willowbrook State School in New York in the 1960's (5,6). Development of serodiagnostic tests for serum or type B hepatitis was greatly accelerated by the serendipitous discovery in 1964 by Blumberg of the "Australia antigen" which subsequently proved to be the hepatitis B surface antigen (7).

With the final isolation and characterization of hepatitis A and B viruses and concomitant development of specific serodiagnostic tests for each, the simple concept of only two forms of viral hepatitis was shattered by the appreciation of non-A, non-B forms of viral hepatitis. By 1975, it was well established that another form of "serum" hepatitis, a putative "hepatitis C", was the major cause of post-transfusion hepatitis (8,9). By 1980, it was appreciated that a significant fraction of routine or fulminant cases of community acquired hepatitis in the U.S. was caused by neither the hepatitis A virus (HAV) or the hepatitis B virus (HBV) (2,3,10,11). An enteric form of non-A, non-B hepatitis (NANB) was also described in 1980 following retrospective analysis of several epidemics in India (12). In the midst of studies that attempted to sort out the various epidemiologic forms of NANB hepatitis, Rizzetto reported the presence of an additional antigen in the livers of Italian patients with chronic HBV (13). This antigen subsequently proved to be the capsid protein of the hepatitis delta virus (HDV), a defective virus capable only of replicating in hepatocytes infected with HBV.

After many failed attempts, the major NANB agents, hepatitis C virus (HCV) and hepatitis E virus (HEV) were isolated and their genomes cloned and sequenced in 1989 and 1990 respectively (14,15). Although the initial serodiagnostic tests available for characterizing these agents were of suboptimal sensitivity, it quickly became apparent that HCV was the cause of the vast majority if not nearly all clinically significant episodes of post-transfusion NANB hepatitis. Similarly, HEV was found to account for most episodes of epidemic jaundice previously classified as enteric NANB. With development of improved second generation serodiagnostic tests for HCV, it was estimated by some authorities that up to 95% of acute viral hepatitis

cases could be accurately attributed to one of the five named hepatitis viruses (3). The term non-A, non-B hepatitis was dropped from the index medicus and was replaced by the more specific terms hepatitis C and hepatitis E. In contrast to the focus on the confusing entity of NANB hepatitis which characterized much of the viral hepatitis literature in the late 1970's and early 1980's, a major fraction of viral hepatitis publications since 1989 have focused on characterization of the hepatitis C virus, the protean manifestations of chronic HCV infection and the development of therapies for this extremely common disease.

However, in the midst of an explosion of new knowledge regarding both HCV and the other named hepatitis viruses, it has become apparent over the last 2-3 years that several distinct NANB disease entities cannot be attributed to infection with HCV or HEV (3). Whereas some of these syndromes appear to be either extremely rare or of little clinical significance, others such as the syndrome of fulminant NANB hepatitis are in many areas of the world the most common cause of fatal acute hepatitis.

Post-Transfusion non-A,B,C:

The initial recognition of NANB viral hepatitis was prompted by the observation of Prince, et al in 1974 that the majority of cases of post-transfusion hepatitis could not be attributed to hepatitis B infections (8). Shortly thereafter, Feinstein, et al described 22 patients at the NIH with post-transfusion hepatitis that was not associated with serologic evidence of hepatitis A or B infection (9). This NANB hepatitis was transmitted to chimpanzees as early as 1978 but a responsible virus evaded isolation until 1989 when Houghton and colleagues at Chiron Corp. in collaboration with Bradley and colleagues from the CDC successfully reverse transcribed, cloned and sequenced the HCV genome (2,3,14). In the interim, a variety of observations suggested that there might be more than one post-transfusion NANB virus.

Multiple apparent episodes of NANB hepatitis were observed in some individuals (16). In addition, a syndrome of short incubation (4-19 days incubation) NANB post-transfusion hepatitis was observed in hemophiliacs that appeared epidemiologically distinct from the longer incubation (average ~ 12 weeks) of typical post-transfusion NANB (17,18). Chimpanzees who appeared to recover from one episode of NANB hepatitis were observed to develop another episode of apparent acute NANB hepatitis when challenged with another lot of infectious sera but not when challenged with another aliquot of the original inoculum (19-21). Finally, isolation of two physicochemically distinct viral strains, a chloroform resistant and a chloroform resistant strain was reported (22).

Following isolation and characterization of the chloroform sensitive NANB strain, HCV, many of the early observations suggesting multiple NANB strains of post-transfusion hepatitis were found to be related to the unique biology of the hepatitis

C virus. HAV or HBV infections in immunocompetent humans typically confer long-lived immunity to subsequent infection by any isolate of the same virus. HCV is an RNA virus with extensive genomic sequence variability. Reinfection of humans or chimpanzees has been observed not only after challenge with a second HCV strain but also after challenge with an identical inoculum (23-25). The biochemical manifestations of reinfection with the same or nearly identical HCV strains may be much less severe than initial episodes. However, after reinfection with more disparate HCV strains, second infections may be more severe than primary episodes. In addition, the incubation interval between second infections and histologic evidence of hepatitis may be quite short (23). These characteristics of HCV appear to explain most of the previous observations related to multiple episodes of post-transfusion NANB hepatitis.

With respect to biophysically distinct forms of post-transfusion NANB viruses, the same lot of factor VII concentrate that was the original source of the chloroform resistant and chloroform sensitive types of NANB viruses has been restudied by Purcell and colleagues (22). When chloroform treated material was inoculated into a chimpanzee, no evidence of hepatitis was observed in the recipient chimpanzee. However, when the untreated material was infused, typical hepatitis C infection was observed (22). In light of this failure to reproduce the original observation, no good explanation for the earlier episodes of hepatitis after chloroform treatment can be offered other than to speculate that residual HCV viral particles remaining after earlier chloroform treatment may have caused the apparent second type of NANB hepatitis (22).

With development of the first anti-HCV test, it was rapidly appreciated that most episodes of NANB post-transfusion hepatitis were associated with development of anti-HCV and that most anti-HCV (+) episodes progressed to chronic hepatitis. In contrast, anti-HCV negative episodes were noted to be less likely to progress to chronic disease. These first generation assays which were based on a single recombinant HCV antigen (the C-100 polypeptide from the NS-4 region of the HCV genome) were subsequently found to have only 80-90% sensitivity in detecting chronic HCV with even lower rates of sensitivity early in the course of acute HCV. With development of second and now third generation anti-HCV assays that incorporate multiple HCV antigens along with use of sensitive PCR based assays for presence of HCV RNA, an increasing fraction of patients with post-transfusion NANB hepatitis have been found to exhibit seroconversions indicative of HCV infection and/or to have circulating HCV RNA in conjunction with biochemical evidence of hepatitis (reviewed in ref. 26).

The results of retrospective analysis of two major post-transfusion hepatitis studies (26-28) is detailed in Table 1. Both studies used relatively broad criteria to define post-transfusion hepatitis. For this reason many patients with minimal biochemical evidence of liver disease were included. The inclusion of a control group

Table 1

Contribution of HCV to NANB Transfusion-Associated Hepatitis

	NIH Study (Ref. 26,28)		U.S. TTV Study (Ref. #27)			
	Transfusion Recipients with Hepatitis		Controls (N=1232) with Hepatitis	Transfusion Recipients (N=1232) with Hepatitis		
	anti-HCV (-), HCV RNA (-)	anti-HCV (+) &/or HCV RNA (+)	all anti-HCV (-)	No anti-HCV	anti-HCV (+) 1st and 2nd Gen. Assay	anti-HCV (+) 2nd Gen. Assay only
# Patients with Hepatitis*	12	86	37	44	51	16
Peak ALT (Mean-NIH, Med-TTV)	302	708	144	156	604	466
Jaundice	0%	30%	0%	0%	24%	20%
Chronic Hepatitis**	17%	70%	6%	10%	59%	54%

* Post-transfusion hepatitis defined in NIH study as two ALT > 2X ↑, at least one ALT > 2.5X ↑; defined in TTV study as two ↑ ALT values within 3-17 day span with one ALT > 2X↑.

** Indeterminate patients without adequate follow-up deleted from calculation of % chronicity.

(hospitalized patients who never received transfusions) in the TTV study demonstrated that many such episodes were not associated with transfusion. Thus, in addition to the 111 of 1230 (9%) transfused patients who met ALT criteria for post-transfusion hepatitis, a surprising 3% (37/1232) of controls also met these ALT criteria for hepatitis. However, fully 60% (67) of the transfusion associated hepatitis cases were marked by an anti-HCV seroconversion, whereas none of the control hepatitis cases developed anti-HCV. Of note the anti-HCV (-) cases of putative post-transfusion hepatitis in both the TTV and NIH studies had characteristics more typical of the mild hepatitis seen in the TTV controls. Such anti-HCV (-) cases tended to have significantly lower peak ALT levels and were less likely to become jaundiced or progress to chronic liver disease. When the TTV investigators took the liberty of adjusting their incidence of transfusion associated hepatitis for "background" rates in the control patients, they estimated that at least 91% of transfusion associated hepatitis cases were associated with markers of HCV infection (27). The 2nd generation anti-HCV screening assays employed in this study are known to fail to detect up to 10% of acute or chronic HCV infections detected by HCV RNA assays. Therefore, it is possible to conclude from these calculations that all transfusion transmitted NANB hepatitis was related to HCV infection. Implicit in this assumption, however, is that the anti-HCV (-), seemingly mild, transient episodes of ALT elevation seen in up to 3.6% of transfusion recipients indeed represent the expected background incidence of ALT abnormalities and are not related to a transmissible agent.

A more recent study of post-transfusion, non-A,B,C hepatitis, suggests that the above assumptions may be overly optimistic (29,30). In this prospective study of surgery patients at a single hospital in Paris, recipients of autologous blood transfusions were used as a control group for recipients of homologous transfusions from donors screened initially only for HBsAg, anti-HIV and surrogate markers of HCV (anti-HBc, ALT > 2X↑). Midway through the study 1st generation anti-HCV (anti-C-100) screening of blood donors was added. Post-transfusion hepatitis was defined as sustained 2-fold increase in ALT values (2 values at least 1 week apart > 2X upper limit of normal) occurring between 4 and 24 weeks post-transfusion. As detailed in Table 2, recipients of allogeneic donor blood developed a significantly higher total incidence of NANB post-transfusion hepatitis as well as a greater frequency of anti-HCV (-) episodes of transfusion associated hepatitis than did recipients of autologous blood or non-transfused surgery patients. The homologous blood recipients with apparent NANB post-transfusion hepatitis were also assessed by PCR based assays for presence of HCV RNA or HBV DNA. Two proved to be HBV DNA (+) despite absence of HBsAg, anti-HBc or anti-HBs but all were HCV RNA (-). HCV RNA was readily detected in the sera of all 3 cases of anti-HCV (+) post-transfusion hepatitis. In this study, as in previous studies, the severity of ALT elevations was greater in anti-HCV (+) cases of post-transfusion hepatitis (mean 14.2 fold peak elevation) than in anti-HCV (-) cases (mean 4.3 fold peak elevation) and only 1 anti-HCV (-) patient progressed to chronic disease. Of note, additional evaluation of donors revealed that 6/16 cases of non-C post-transfusion hepatitis were associated with at least one donor with mild ALT elevations between 1 and 2 fold the upper limit of normal (29). As risk for developing non-A,B,C transfusion associated hepatitis was only statistically associated with receipt of homologous transfusions and not with age, sex, hepatotoxic drug consumption, type of surgery or length of hospitalization, it was concluded that these episodes were most likely associated with an as yet unidentified transmissible agent (29,30).

Table 2

Association of non-A,B,C Hepatitis with Homologous Blood Transfusion (29,30)

	Homologous Blood, no anti-HCV Screen	Homologous, anti-HCV (-) Blood	Autologous Blood	Untransfused Surgery Patients
Number of Patients	118	63	90	64
Episodes HCV	2 (1.7%)	1 (1.6%)	0	0
Episodes non-A, non-C, HBsAg (-) Hepatitis	15 (12%)*	4 (6.3%)*	1 (1.1%)	1 (1.6%)

* Two homologous blood recipients with hepatitis found to have serum HBV DNA.

Fulminant non-ABCDE Hepatitis (Hepatitis F?):

Shortly after discovery of the syndrome of post-transfusion NANB hepatitis, it came to be appreciated that a significant fraction of patients presenting with fulminant hepatic failure secondary to apparent viral hepatitis exhibited no serologic markers of HAV or HBV infections (10,11,31). The relative frequency of HAV, HBV and putative NANB infections among cases of fatal or fulminant hepatitis in two large patient series are detailed in tables 3 and 4. The data in table 3 were accumulated over a 12.5 year interval between January 1, 1971 and June 30, 1983 at an infectious disease hospital in Melbourne, Australia (31). Stored sera from hepatitis patients were tested for presence of IgM anti-HAV and HBsAg. Sera from fatal cases was also assessed for presence of anti-HBc, IgM anti-HBc and antibodies to EBV and CMV. It was found that HAV infections were associated with a very low (0.14%) case fatality rate. The case fatality rate among cases associated with markers of HBV infection was 0.84%, a rate comparable to that reported in other series. However, NANB hepatitis was associated with a significantly higher fatality rate (2.3%). Even more striking was an analysis of the epidemiology of fatal cases. Among fatal HBV cases, a relatively high fraction (9 of 19) were associated with a recent blood transfusion thought to be the route of transmission (31). In contrast, only 1 or 24 NANB cases followed a blood transfusion and none were associated with intravenous drug use despite the frequency of this risk factor among the nonfatal episodes of NANB hepatitis (31).

Table 3

Incidence of Acute HAV, HBV and NANB Hepatitis
in Melbourne, Australia from 1971-1983

	Total # of Cases	# of Fatal cases	Case Fatality Rate
Hepatitis A	2174	3	0.14%
Hepatitis B	2253	19	0.84%
NANB Hepatitis	1050	24	2.29%

In Table 4, the apparent etiologies of fulminant hepatic failure in patients with viral hepatitis referred to the King's College Hospital from 1973 - March 1993 are summarized (32). The finding that NANB hepatitis appeared to cause as many cases of fulminant hepatic failure as HAV and HBV combined is especially striking since an epidemiologic study conducted during this same era at another hospital in London suggested that HAV accounted for 51% of acute hepatitis cases, HBV for 34% of cases and putative NANB agents for only 13% of cases of apparent acute viral

hepatitis (33). Of additional note, fulminant or subfulminant NANB hepatitis was noted to have a significantly higher mortality rate than fulminant HAV or HBV cases(34).

Table 4
Classification of Acute Hepatitis of Apparent Viral Etiology Progressing to Grade III-IV Hepatic Encephalopathy at King's College Hospital, London

	Cases, 1973 - 1993		Survival, 1982-1985
	# of Cases	Percent	
Hepatitis A	60	16.6%	66.7%
Hepatitis B	94	26.0%	38.9%
NANB Hepatitis	201	55.7%	20.0%
Other	6	1.7%	N.S.

N.S. = Not stated.

Studies of fulminant hepatic failure conducted in the U.S. during the 1970's found that the majority of cases appeared to be secondary to Hepatitis B (11,35). However, following the wide spread availability of liver transplantation as therapy for this disease in the 1980's, most U.S. transplant centers found that fulminant NANB or fulminant hepatitis of uncertain etiology was a more common indication for emergent liver transplantation than was fulminant hepatitis B (36-38). Following availability of serologic and PCR based assays for HCV and HEV infections, numerous investigators based at liver transplant or liver disease referral centers began to look for evidence of HCV or HEV infection in cases of fulminant or subfulminant NANB hepatitis. The results of such studies are summarized in Table 5.

The results detailed in Table 5 indicate that neither hepatitis C nor Hepatitis E is a common cause of fulminant NANB hepatitis in the U.S. or Europe. The relatively infrequent nature of fulminant hepatitis C correlates well with previous observations suggesting that post-transfusion NANB hepatitis rarely progresses to hepatic encephalopathy (31,50,51). Enteric NANB hepatitis, subsequently shown to be hepatitis E, has been frequently associated with progression to encephalopathy and death. However, acute hepatitis E has rarely been identified in the U.S. or Europe other than in cases related to recent travel to endemic areas (52,52). Indeed, 6 of the 8 patients in the London series with fulminant hepatitis E (44) had a recent history of foreign travel. Thus, it is perhaps not surprising that HEV infection is a rare cause of fulminant hepatitis in the U.S. and Europe.

Table 5
Hepatitis C and E are Rare Causes of Fulminant or Subfulminant Hepatic Failure in the U.S. and Europe

First Author / Referral Center	Number of NANB Cases[a] Examined	Number with HCV Markers	Number with HEV Markers	Number with HBV DNA
Wright (39,40), San Francisco	17	0	N.T.	7[b]
Feray (41), Clichy & Villejuif, France	23	1[c]	0	1[d]
Liang (42), Miami	17	2[e]	0	0
Sallie (43,44), London	42	0	8	0[f]
Theilmann (44b), Heidelberg	8	2	0	0
Laskus/Kuwada(45,46), Rochester, Minn.	8	0	0	0
Combined U.S. / Europe	***115***	***5/115 (4.4%)***	***8/98 (8.2%)***	***8/115 (7.0%)***
Yanagi (47), Kanazawa, Japan	7	4[g]	N.T.	N.T.
Yoshiba (48), Yokohama City, Japan	23	13[h]	N.T.	N.T.
Chu (49), Taipei, Taiwan	11	5[i]	1[j]	0[d]
Combined, Asia	***41***	***22/41 (53.7%)***	***1/11 (9.1%)***	***0***

[a] NANB defined as IgM anti-HAV (-), HBsAg (-), and IgM anti-HBc (-).
[b] Found in explant liver in 6 patients. One additional patient with no explant tissue to examine was found to have post-transplant serum markers of HBV infection as did 4/6 patients with HBV DNA in liver pre-transplant.
[c] One patient anti-HCV (+) but serum HCV RNA (-). HCV RNA was found in serum of 7/17 HBsAg (+) patients with fulminant hepatic failure; all anti-HCV (-).
[d] Only serum samples screened for HBV DNA in this study.
[e] HCV RNA found in serum of one patient and in liver of another; both anti-HCV (-).
[f] Based on PCR assay for HBV DNA in livers of 45 NANB patients.
[g] Three patients anti-HCV (+), three were serum HCV RNA positive. HCV markers also found in 1/3 IgM anti-HAV (+) patients and 4/8 HBV marker (+) patients.
[h] Eight patients were anti-HCV (+), 12 were serum HCV RNA positive.
[i] Four patients anti-HCV (+), 5 were HCV RNA positive. Serum HCV RNA also found in 1/5 fulminant hepatic failure patients with acute HBV and 12/46 HBsAg carriers with fulminant hepatitis.
[j] Acute HEV also diagnosed in 1/5 acute HBV cases and 4/46 HBsAg carriers with fulminant hepatitis.

The basis for an apparently higher frequency of fulminant hepatitis C in Japan and Taiwan is unclear. As the HCV genotypes common in Japan are in many cases significantly different from those commonly found in the U.S. or Europe, a strain difference in virulence has been offered as one potential explanation for this discrepancy (48,55). To some extent this argument has been supported by the high frequency of Okamoto Type II and III strains among Japanese cases of fulminant hepatic failure (48). Both strains are quite rare in the U.S. where the Okamoto Type I strain is prevalent. However, a recent prospective study of post-transfusion hepatitis in Japan has estimated that the incidence of fulminant post-transfusion NANB hepatitis (and by inference post-transfusion HCV, the cause of most cases) is less than 0.2% (51). The carrier rate for chronic HCV in Japan is among the highest of any industrialized country and it has been suggested that many HCV marker positive cases of fulminant hepatitis in Japan may represent chronic HCV carriers with a superimposed seronegative hepatitis (48,55). A precedent for this mechanism is found in the French and Taiwanese experience in which many HBsAg (+) patients with fulminant presentations appeared to be chronic carriers with superimposed acute HCV (41,49) or HEV (49) infections. An additional argument in favor of this hypothesis is the observation that most French cases of acute HCV leading to fulminant decompensation in HBsAg (+) patients were detected only by HCV RNA assays. The antibody response to HCV develops later in the course of HCV than HAV or HBV (54) and the more frequent concomitant presence of (+) anti-HCV and HCV RNA assays in the Japanese patient series has been used to argue that these patients may be chronic carriers with a second cause of acute hepatitis (55). However, with more recently developed second generation assays, anti-HCV is frequently detected at time of initial presentation in acute HCV (56-61). Unfortunately IgM assays do not appear useful in distinguishing acute from chronic HCV infections (62) and thus there is no good technique for distinguishing acute form chronic HCV infections in such patients.

Irrespective of the mechanisms underlying the differences in the role of HCV in fulminant hepatic failure in Asian versus Western countries, the present evidence argues persuasively that a significant fraction of cases of fulminant hepatitis appear unrelated to infection by any of the 5 well characterized hepatitis viruses. These seronegative cases of fulminant hepatic failure have previously been attributed to viral infections because they typically present with the same symptoms, biochemical abnormalities and histologic manifestations as fulminant hepatitis cases associated with documented hepatitis virus infections (34,36). However, there is little additional epidemiologic data to argue that such cases are indeed related to a virus or other transmissible agent. Multiple investigators have commented upon the absence of parenteral risk factors in such patients (31,39,45) and no association with epidemics or clusters of hepatitis cases has been reported. The only direct evidence suggesting involvement of an infectious agent has been reported in 9 patients with fulminant hepatic failure who presented to the King's College Liver Failure Unit. Fagan, et al (63) reported the presence of 60-70 nm enveloped Toga virus-like particles bin livers of these patients. In 5 of these patients, acute hepatic failure recurred within 7 days

after liver transplantation. The liver allografts in these patients were characterized by severe hemorrhagic necrosis and presence of similar virus like particles. However, other transplant centers have yet to report similar electron microscopic findings or rapid recurrence of fulminant hepatic failure in patients receiving liver transplants for fulminant NANB hepatitis. Nevertheless, it should be noted that although HBV and HCV infections frequently recur after liver transplantation, such post-transplant infections rarely manifest unique biochemical or histologic features that clearly distinguish them from allograft rejection or a host of other opportunistic viral or other infections. Only with use of specific serologic and molecular assays has the true incidence of these recurrent infections been appreciated.

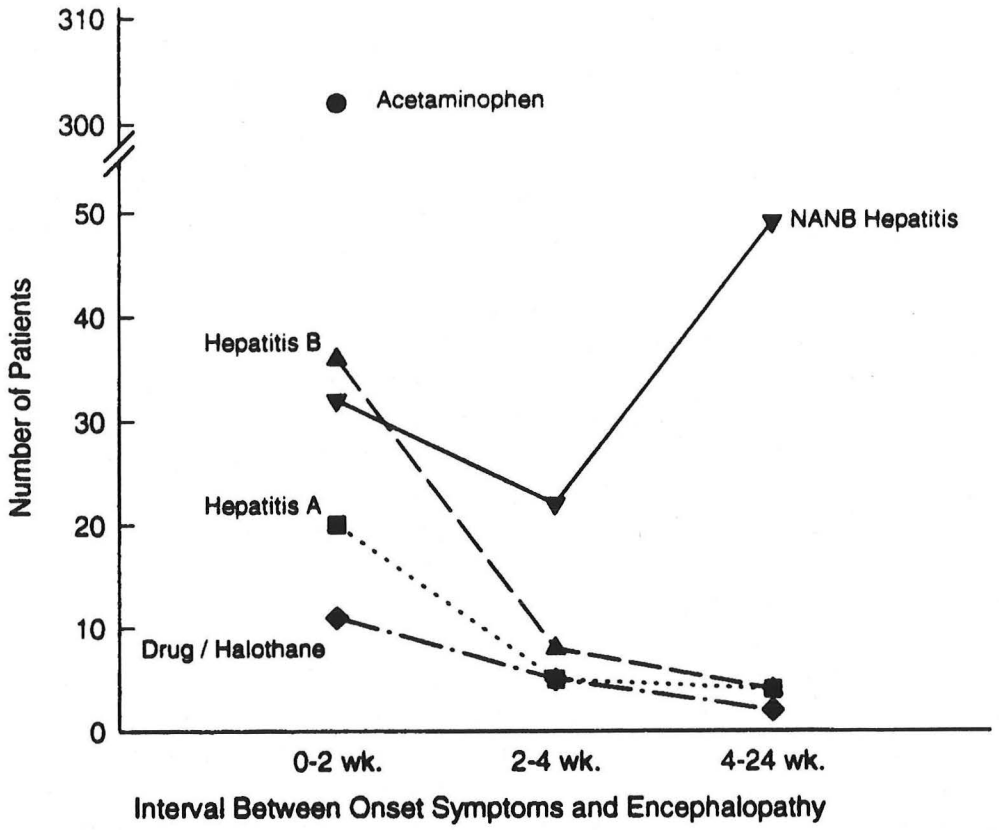
Despite ambiguities regarding its etiology, the syndrome of fulminant NANB hepatitis is characterized by a number of features that tend to distinguish it from other viral or toxic causes of fulminant hepatic failure. These characteristics are summarized in table 6. Fulminant Hepatitis B and fulminant NANB hepatitis differ significantly in mean time interval between onset of either first symptoms or jaundice and development of hepatic encephalopathy (48,64,65,67). An analysis of Japanese patients (48) with fulminant hepatitis has found that both seronegative cases and those with HCV markers are characterized by a more insidious course with delayed onset of hepatic encephalopathy (mean 29 and 34 days after onset) and multiple aminotransferase peaks. In contrast fulminant HBV was usually associated with an early single aminotransferase peak and rapid progression (mean 9 days) to hepatic encephalopathy.

Table 6
Distinguishing Clinical Characteristics of
Fulminant Seronegative Hepatitis

Characteristic	Reference
Prolonged interval between onset symptoms and development of encephalopathy	48,64,65,67
Absence of parenteral risk factors	31,39,45,64
Multiple Transaminase peaks	48
High mortality rate	34,66
Association with aplastic anemia	68,69

Analysis of the largest single published series of fulminant hepatitis cases has suggested that there is actually a bimodal distribution in rate of progression to hepatic encephalopathy in cases of fulminant seronegative hepatitis (65,67,figure 1). Unlike

Figure 1



fulminant acetaminophen hepatotoxicity in which encephalopathy invariably develops within 7 days of onset of symptoms, or most cases of fulminant hepatitis A, hepatitis B or drug toxicity in which the interval between onset of symptoms and development of hepatic encephalopathy is usually < 2 weeks (67, figure 2), encephalopathy often does not develop until weeks or months after the onset of symptoms in patients with NANB hepatitis (65,67).

When the NANB patients in the King's College Hospital series with late onset hepatic failure (LOHF, defined as hepatic encephalopathy developing 8-24 weeks after onset of symptoms of hepatitis) were compared to NANB patients with fulminant hepatic failure (FHF, encephalopathy within 8 weeks of onset of symptoms), the LOHF patients were found to differ from FHF patients in mean age (44.5 yrs in LOHF vs. 25.5 yrs. in FHF) and were found to be more likely to develop ascites and renal failure and less likely to develop cerebral edema during their hospital course. Both groups had similar acute histologic abnormalities and a high mortality rate. However, 3/5 long term, non-transplanted survivors of LOHF continued to have chronic hepatitis with piecemeal necrosis (chronic active hepatitis) on liver biopsy more than 1 year after initial presentation. Development of chronic liver disease following FHF was not reported in this series (65). In 8 of 39 LOHF patients tested (~20%) autoantibodies (ANA, ASMA) were detected and 49% had elevated IgG levels but this frequency of autoantibodies or hyperglobulinemia was not statistically different from the incidence in FHF patients. The majority of LOHF patients were treated with corticosteroids and

the survival rate among those receiving corticosteroids (7/30, 23%) was higher than among untreated patients (0/16) but this difference was not statistically significant ($p = 0.053$).

An another rare but striking complication that has been associated with fulminant NANB hepatitis is the development of aplastic anemia (68). Cases of aplastic anemia associated with a recent episode of acute hepatitis have been reported over a period of several decades (69). Most cases have been found not to be associated with markers of HAV or HBV infection and in a survey of liver transplant centers (68) all cases that occurred after liver transplantation were found to follow transplantation for acute NANB hepatitis (9 cases of aplastic anemia in 32 patients with fulminant NANB, 0 cases in 1463 patients transplanted for other indications). Recently, application of anti-HCV and HCV RNA assays to 28 patients with aplastic anemia and NANB hepatitis has suggested that whereas HCV infection frequently occurs after transfusion in such patients, HCV markers are absent at time of initial diagnosis prior to initiation of blood product infusions (69). Of note, the age range of patients with fulminant NANB and associated aplastic anemia after liver transplantation (5-20 years, mean 9 yrs) and the mean time interval between onset of hepatitis symptoms and transplantation for hepatic coma ($\leq$ 4 weeks in 7/9 patients) is distinctly different from that reported for patients with LOHF (65,68). It is therefore unclear whether there are distinct clinical and etiologic syndromes among patients with fulminant non-ABCDE hepatitis or if alternatively, these differences reflect age differences in response to a common etiologic agent.

Syncytial Giant-Cell Hepatitis (Hepatitis G?):

In 1992 Phillips, Purcell and colleagues from the University of Toronto and the NIH (70), described 10 patients with severe hepatitis characterized histologically by large syncytial hepatocytes containing intracytoplasmic structures thought to be consistent with paramyxoviral nucleocapsids. Four of these patients presented with subacute hepatic failure and the other six presented with a clinical presentation consistent with chronic active hepatitis (5 initial diagnosed as autoimmune hepatitis). All patients either died of liver failure or underwent liver transplantation. Histopathologic findings in the livers of these patients included both features of chronic hepatitis and bridging necrosis and large syncytial cells containing up to 30 nuclei and infiltrated by neutrophils. In 8 of 10 patients, electron microscopy revealed the presence of intracytoplasmic spherical particles 150-250 nm in diameter and filamentous structures containing 14-17 particles with peripheral spikes thought consistent with the previously described structure of paramyxoviruses.

Liver tissue from one of the Toronto patients with syncytial giant cell hepatitis was frozen, ground up, suspended in balanced salt solution and infused into two chimpanzees. Neither animal developed biochemical or histologic evidence of hepatitis

but one developed antibodies reactive to two paramyxoviruses, measles and parainfluenza 4, in complement fixation assays. Hemagglutinating antibodies to sheep red blood cells were also found in the convalescent sera from this chimpanzee. In light of the electron microscopic findings in 8 of the patients, the presence of autoimmune hemolytic anemia in 2 patients and the known propensity for giant cell formation in other tissues infected by paramyxoviruses, the authors proposed that this was a new paramyxovirus mediated form of hepatitis.

However, following the report by Phillips, et al, a number of other groups performed retrospective analysis of their liver biopsy specimens looking for cases of syncytial giant cell hepatitis. Most such specimens could not be evaluated by electron microscopy. However, in 5 cases in which electron microscopy was performed by two different groups of investigators (73,76) no viral particles could be found. Some investigators reported finding "giant cells" in patients with liver disease of defined etiology and suggested that since giant cell formation is a common histologic finding in infants with heterogeneous causes of liver disease, such syncytial giant cell formation in adults, while rare (overall present in ~ 1/200 diseased livers), might merely represent a non-specific pathologic response.

However, review of multiple small series of cases of giant cell hepatitis, including several that were published prior to the report from Phillips, et al, reveals a number of clinical features that appear to be present in multiple case series (see table 7). Despite the claims by some authors that most such patients have well defined etiologies for their liver disease, the most common "etiology" is autoimmune hepatitis, an entity with at best loosely defined diagnostic criteria (77). In contrast to the report of Phillips, et al, some patients with syncytial giant cells on initial liver biopsy appear to respond to corticosteroid therapy and/or to have a self-limited disease that eventually resolves. However, as detailed in table 7, despite the heterogeneity of mortality and steroid response rates, it is clear that most such patients have a clinically severe illness (91% of cases described as histologically or clinically severe) that is often associated with the autoantibodies frequently present in autoimmune hepatitis (37% with ANA or ASMA) or with antibodies reactive with autologous red blood cells (25% of cases).

Although other investigators have not been able to provide evidence supportive of a paramyxovirus as the specific cause of giant cell hepatitis, a recent report by Pappo, et al from the University of Pittsburgh provides evidence consistent with a transmissible agent. In this report, syncytial giant cells were found in 14 of 3416 native livers removed from patients undergoing allogeneic liver transplantation. In reviewing the post-transplant biopsies, recurrent syncytial giant cell formation was observed in 5 cases. In addition, two additional patients developed giant cell hepatitis, *de novo*, after liver transplantation. Two of the five patients with recurrent giant cell hepatitis died and two required repeat transplantation because of recurrent liver failure. One of the latter patients developed giant cell hepatitis in a second allograft.

Table 7

Characteristics of Patients with Giant-Cell Hepatitis

	Phillips, et al (70)	Bernard, et al (71)	Thaler (72)	Devaney, et al (73)	Lau, et al (74)	Dienes, et al (75)	Pappo, et al (76)	***Combined***
Number of cases:	10	4	6	20	12	16	7	-
Age Range:	0.4-42 yr.	0.5-2 yr.	12-74 yr.	2-80 yr.	12-72	38.5 yr.	0.3-54 yr.	-
Prevalence:	N.S.	N.S.	6/2423	N.S.	12/12,000	16/1467	14/3416	***~1/200***
Male/Female:	5/5	3/1	2/4	10/9	9/3	8/8	2/5	***53% / 47%***
Autoantibodies:	5/10	0/4	1/6	7/17	3/12	8/16	N.S.	***37%***
Coomb's (+) HA:	2/10	4/4	0/6	3/17	N.S.	N.S.	2/7	***25%***
Death or Transplant:	10/10	3/4	0/6	4/17	6/12	N.S.	14/14	***54%***
"Severe":	10/10	4/4	5/6	N.S.	8/12	16/16	7/7	***91%***
Steroid Responsive:	0/10	1/4	3/3	6/17	1/2	12/16	N.S.	***44%***
Resolved:	0/10	N.S.	5/6	6/17	2/12	N.S.	0/7	***25%***

While these authors could not find viral particles in livers with giant cell hepatitis, they did identify human papilloma virus 6 by PCR analysis in liver tissue from 3 pre-transplant and 4 post-transplant livers.

Thus while syncytial giant cell hepatitis fortunately seems to be a very rare entity, many such patients have severe, life threatening disease. The specificity of this histologic finding is still uncertain, but evidence for transmissibility has been sufficient for some reviewers to propose that it might represent a new "hepatitis G" (70,78).

Enteric non-ABCDE:

Following isolation and characterization of hepatitis E (15), recombinant HEV polypeptides prepared from the genetic sequence of several geographically diverse HEV isolates were used to establish anti-HEV assays. Such assays have proven useful in establishing prevalence of HEV infections in many areas of the world (52,53,80, 81). However, a recent careful re-analysis of water-borne epidemics of hepatitis in India (79) has suggested that all cases of hepatitis in a large (307 case) epidemic in the Andaman Islands as well as a significant fraction of cases in several other epidemics appear not to be associated with serologic markers of HEV, HAV, HBV or HCV infection. The Andaman Island epidemic was associated with all classic signs and symptoms of viral hepatitis but included a much higher incidence of icteric hepatitis in children than has been described in epidemics of HEV (79). Sequence analysis of

HEV isolates from the 15 HEV epidemics examined in parallel in this study indicated that they were all closely related. Moreover, even more distantly related strains causing epidemics in Mexico are known to cause antibody responses detected by the assays used. Thus the authors conclude that there is likely to be at least one additional enteric hepatitis virus.

Community Acquired or Sporadic non-ABCDE Hepatitis:

Early in the evolution of anti-HCV testing, a higher frequency of anti-HCV reactivity was noted in chronic non-A, non-B hepatitis than in acute non-A, non-B hepatitis (82). This discrepancy was largely attributed to the delayed nature of seroconversion to the C-100 antigen used in this original assay. Indeed, as additional HCV antigens have been added to diagnostic assays it has been observed that seroconversions to C-100 epitopes occur significantly later than seroconversions to other HCV antigens (54). However, in earlier studies using first generation anti-HCV assays as well as later studies using second generation ELISA assays and/or supplemental PCR assays (56,58,60, 83-85), it has been noted that anti-HCV markers are less likely to be found in acute NANB hepatitis cases not associated with parenteral risk factors than in cases associated with recent transfusion or parenteral drug use. Finally with application of both antibody and rtPCR RNA assays for both HCV and HEV, it has become apparent that a significant fraction of sporadic, community acquired cases of NANB hepatitis can not be attributed to either HCV or HEV infection.

As detailed in table 8, the fraction of sporadic (non-parenteral) NANB cases without ascribed etiology varies from 33% to 85% in different series. The relative prevalence of Hepatitis A and B also varies widely from country to country and among different regions of large countries such as the U.S. (87). Based on CDC data suggesting that NANB hepatitis accounts for ~26% of all cases of acute hepatitis, the data detailed in table 8 would suggest that ~4.8% (18.5% of 25%) of acute hepatitis cases in the CDC's sentinel counties and ~8.3% of cases in Dallas, Texas cannot currently be attributed to any of the five named hepatitis viruses. The study from Barcelona, Spain summarized in table 8 (85), concurrently assessed the frequency of acute HAV, HBV, HBV + HDV, HCV and HEV and came to the conclusion that fully 20% of Spanish cases of acute hepatitis remain seronegative.

Such seronegative cases of acute hepatitis appear largely unrelated to parenteral risk factors in all studies (table 8). In addition, in both the CDC study of NANB hepatitis conducted in 5 selected sentinel counties across the U.S. and in an ongoing prospective analysis of acute NANB cases seen at UT Southwestern Medical Center in Dallas (83), major ethnic differences between patients with acute HCV and those with acute non-ABCDE hepatitis have been noted. In both studies, patients with acute HCV were more likely to be white than were patients with acute non-ABCDE. In the Dallas series (83), the ethnic background of the acute non-ABCDE cases was

Table 8

Contribution of Hepatitis C, Hepatitis E and non-ABCDE Hepatitis to Community Acquired cases of NANB Hepatitis

Site:	All NANB Cases			Parenteral Risks			Sporadic		
	#	HCV	HEV	#	HCV	HEV	#	HCV	HEV
U.S, CDC[1] (56)	130	82%	N.E.	66	95%	N.E.	64	67%	N.E.
Los Angeles[2] (58)	231	79%	N.E.	135	91%	N.E.	96	61%	N.E.
Dallas[3] (83)	103	65%	2.9%	57	95%	0	46	28%	7%
India[1] (84)	-	-	-	-	-	-	89	12%	44%
Spain[1] (85)	138	54%	0	75	87%	0	63	14%	0
Japan[1] (60)	49	65%	N.E .	29	83%	N.E.	20	40%	N.E.

N.E. = Not examined.
[1] Employed both second generation anti-HCV and HCV RNA testing.
[2] Employed second generation anti-HCV testing of acute (0-2 wk.) sera.
[3] Employed second and third generation anti-HCV testing.

not statistically different than that of the general patient population at Parkland Memorial Hospital where most of the patients initially presented whereas white patients were significantly over represented among the patients with acute HCV. The CDC study also found that patients with seronegative hepatitis were older (46% ≥40 yrs of age) than patients with acute HCV (24% ≥ 40 yrs of age.

In both an older study of acute hepatitis in Melbourne, Australia (31) and the ongoing study of acute NANB hepatitis at this institution, a significant incidence of fatal hepatitis has been noted among patients with acute NANB hepatitis (2.9% in Australia, 4/103 = 3.9% with death/transplantation in the Dallas series). One of the patients progressing to FHF and death in the ongoing study at this institution had convalescent antibodies against HEV antigens whereas the other three were anti-HCV (-), anti-HEV (-) and HCV RNA negative. In addition, the patients with acute non-ABCDE hepatitis seen in this institution have been found to have other biochemical markers of severity including higher peak total bilirubin levels, greater likelihood of prothrombin time prolongation and greater degrees of hypoalbuminemia than has been seen among cases of acute HCV (83). In contrast, among the somewhat smaller number of non-A,B,C cases detailed in the CDC study no fulminant cases are mentioned and the only biochemical difference noted was a lower peak ALT level

among seronegative patients (56). A recent analysis of patients with sporadic acute NANB hepatitis in Athens, Greece has found that patients with non-ABCDE hepatitis have histologically more severe acute disease than is seen among patients with acute HCV. In addition, while acute HCV is significantly more likely to progress to chronic liver disease (~60% develop chronic hepatitis, ref. 56,88), a smaller but significant fraction of anti-HCV (-), HCV RNA negative cases (26-29%, ref. 56,88) also appear to progress to chronic liver disease which may be histologically severe.

Summary and Recommendations:

Despite identification and characterization of two new hepatitis viruses and development of serodiagnostic tests for these agents, a small fraction of cases of acute hepatitis continue to evade classification. While many such cases appear to represent mild self-limited illness, a subset of these patients progress to hepatic failure and death or liver transplant and others appear to progress to chronic hepatitis. It is at present unclear whether these episodes of seronegative or "non-ABCDE" hepatitis are related to infections by additional hepatitis virus(es), to unidentified hepatotoxins, or to autoimmune or other noninfectious mechanisms. With respect to a practical clinical approach to such cases, it is important to remember that as in the case of the previous term "non-A, non-B hepatitis" present cases of non-ABCDE represent a diagnosis of exclusion and likely encompasses a heterogeneous group of diseases.

The term acute hepatitis refers to a biochemical and histologic syndrome reflecting acute or chronic hepatocellular injury. In studies of post-transfusion hepatitis, investigators have considered all episodes of sustained 2-fold ↑ ALT as representing potential cases of transfusion transmitted viral hepatitis. While this approach has the advantage of identifying even the mildest cases of anicteric viral hepatitis, there are a host of other viral and non-viral diseases that can cause this degree of aminotransferase elevation. Among asymptomatic blood donors with isolated ALT abnormalities, extensive evaluation reveals identifiable causes other than viral hepatitis in the majority of patients with fatty liver disease being the most common single final diagnosis (89,90). Only about 1 in 4 such patients have a clinical and histologic picture suggestive of acute or chronic hepatitis. A significant fraction of these cases can now be attributed to acute or chronic hepatitis C (15% of blood donors rejected for ALT elevation), leaving only about 10% of cases potentially related to other unidentified causes of non-ABCDE hepatitis (90).

Among symptomatic patients with potential acute viral hepatitis, the vast majority have 10-20-fold or higher elevation of aminotransferases (56). In our attempts to prospectively identify potential cases of NANB hepatitis at this institution (83), we have screened patients with a > 10-fold aminotransferase elevation and a symptom complex felt consistent with acute viral hepatitis. Even among patients meeting more stringent biochemical criteria for acute hepatocellular injury, one can anticipate finding patients with drug induced hepatotoxicity, ischemic liver injury or

other systemic infections that also cause liver injury and an apparent "hepatitis". In accumulating the first 33 patients in our local series with apparent non-ABCDE hepatitis (83), we excluded an even greater number of "seronegative" hepatitis cases in which other non-viral causes could be identified. This included not only the expected cases of drug-induced hepatotoxicity, ischemic liver injury or systemic infections but also included a surprising number (12) of cases of choledocholithiasis that presented with 20-30 fold elevations of aminotransferases. In most such patients, a diagnosis of biliary colic was strongly suggested by the clinical history and with the exception of patients with retained common duct stones, all liver enzyme abnormalities resolved within a few days after the initial attack of right upper quadrant pain.

In cases in which a careful review of history, exam, hepatitis serologies, tests for other infectious agents (especially CMV, EBV) and ancillary tests such as an abdominal sonogram are unrevealing of alternative etiologies of liver injury, the clinician faces a good deal of uncertainty in regard to advising and treating the patient. Fortunately by the second or third visit, most patients with hepatitis of defined viral etiology as well as those with apparent acute non-ABCDE hepatitis have begun to improve. If such an illness rapidly and completely resolves, it is not clear that any additional diagnostic evaluation is warranted. In patients with unusually severe or prolonged illnesses the specter of progression to acute liver failure or to chronic liver disease is very real. In such cases, there are several areas in which medical intervention may make a difference in outcome.

First and foremost, in the evaluation of such patients it is important to identify potential drug or toxin exposure, especially in the case of medications that the patient may have continued to take after onset of illness or agents such as acetaminophen in which specific supportive therapies may make a difference in outcome. A careful medication history (including a history of acetaminophen and alcohol use) should be part of the initial interview of any patient with suspected hepatitis. However, in many cases an offending drug is only identified after repeated questioning.

In patients with prolonged episodes of acute hepatitis, it should be recalled that autoimmune hepatitis presents in a significant fraction of patients with an acute presentation initially indistinguishable from that of acute viral hepatitis (77,91). In such patients, corticosteroid therapy can be lifesaving (92). However, the autoantibody markers most commonly associated with autoimmune hepatitis (ANA, ASMA) are unfortunately neither highly specific or sensitive. Both autoantibody responses can be seen in other forms of acute or chronic viral hepatitis (93) and up to 30-40% of patients with corticosteroid responsive chronic hepatitis lack either autoantibody marker (77,94,95). For these reasons, a variety of factors must be taken into account in making a decision to initiate therapy. If therapy is initiated and the patient recovers (responds?), one must reserve judgement regarding need for chronic immunosuppressive therapy until long term followup indicates that the patient indeed

has a chronic relapsing disease consistent with a diagnosis of autoimmune hepatitis.

Finally in patients with biochemical markers of unusually severe disease such as significant coagulopathy (> 3 sec. prolongation of prothrombin time) and/or severe jaundice (bilirubin > 20 mg/dl), the very real possibility of progression to acute liver failure should be considered. Once such a patient develops hepatic encephalopathy, survival in the absence of a liver transplant is 20% or less (34). Thus suitability for liver transplantation should be assessed and prompt referral to an appropriate center should be initiated in an expeditious manner in such patients (36).

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